**Authentication of garlic (*Allium sativum*) supplements using
a *trnL*^{UAA} mini-barcode**

Journal:	Genome
Manuscript ID	gen-2021-0001.R1
Manuscript Type:	Note
Date Submitted by the Author:	30-Apr-2021
Complete List of Authors:	Paradiso, Lydia; New York Botanical Garden, Little, Damon; The New York Botanical Garden, Cullman Program
Keyword:	DNA barcoding, garlic, herbal supplement authentication, Allium, trnL
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

SCHOLARONE™
Manuscripts

1

1 Authentication of garlic (*Allium sativum* L.) supplements using a *trnL*^{UAA} mini-barcode

2

3 Lydia Paradiso*^{1,2}, Damon P. Little^{1,2}

4 *corresponding author: lparadiso@nybg.org

5 ¹The New York Botanical Garden: Bronx, NY, USA. ²The Graduate Center, City University of New York: New

6 York, NY, USA.

Draft

Abstract

Garlic (*Allium sativum*), a widely distributed plant with great cultural and medicinal significance, is one of the most popular Herbal Dietary Supplements (HDS) in Europe and North America. Garlic supplements are consumed for a variety of medicinal applications, including as an antihypertensive, antibacterial, and anticarcinogenic agent. The steady increase in the global HDS market paired with a global patchwork of regulatory frameworks makes the development of assays for authentication of these products increasingly important. A DNA mini-barcode assay was developed using the P6 loop of the plastid *trnL*^{UAA} intron to positively identify *A. sativum* products. Analysis of 43 commercially available garlic HDS produced mini-barcode sequences for 33 supplements, all of which contained detectable amounts of *A. sativum*. The *trnL*^{UAA} P6 mini-barcode can be highly useful for specimen identification, particularly from samples which may contain degraded DNA.

Keywords: DNA barcoding, herbal supplement authentication, garlic, trnL-UAA, *Allium*

Introduction

Garlic (*Allium sativum* L., Amaryllidaceae) is a widely distributed plant which has been cultivated for thousands of years for its culinary and medicinal uses and is currently one of the most widely consumed herbal supplements (HDS) in the USA, Canada, and the EU (Garcia-Alvarez et al. 2014; Singh & Levine 2006; Wu et al. 2014). Its numerous documented medicinal properties include activity as an anticarcinogenic (Seki & Ariga 2006; Liu et al. 2015; Nouroz et al. 2015), antioxidant (Chung 2006; Rahman et al. 2012; Choi et al. 2014), antidiabetic (Ashraf et al. 2011; Padiya & Banerjee 2013; Zhai et al. 2018), antibacterial (Al-Snafi 2015; Hussein et al. 2017), antifungal (Davis 2005; Li et al. 2014), antiparasitic (Abdel-Hafeez et al. 2015; Orengo et al. 2016), and antihypertensive (Ried and Fakler 2014; Rohner et al. 2015; Dubey et al. 2017) agent. These observed properties are imparted by several organosulfur compounds (notably aliin, allicin, ajoenes, and S-allylcysteine [SAC; Batiha et al. 2020]), the abundance of which in a given plant are highly dependent on the choice of cultivar and growing conditions, and further affected by the part of the plant sampled and methods of preservation and preparation (Martins et al. 2016). A surge in the regular use of herbal dietary supplements (HDS) worldwide in recent decades (Messerer et al. 2001; Hirayama et al. 2008; Garcia-Alvarez et al. 2014; American Botanical Council 2020a) has been accompanied by concerns regarding product quality (New York State Attorney General 2015; American Botanical Council 2020b). Classification and subsequent regulation of HDS varies greatly by jurisdiction; some countries classify HDS as

foodstuffs, while others consider them a medicine or drug for regulatory purposes (Prasad Giri et al. 2008; Low et al. 2017). In the USA, HDS are regulated by the Dietary Supplement Health and Education Act of 1994 (DSHEA), which prohibits manufacturers from marketing products that are adulterated or misbranded. Federal regulations require that common names of botanical ingredients be consistent with those standardized in Herbs of Commerce (McGuffin et al. 2000). Under DSHEA, compliance is monitored internally, which can allow illegal adulterated or mislabeled HDS to enter the market. Botanical ingredients may be accidentally or deliberately substituted due to misidentification or economic factors such as cost and availability, and/or contain additional off-label, and therefore illegal, ingredients as fillers (e.g., rice [*Oryza sativa*] or soybean [*Glycine max*]; Newmaster et al. 2013; Little 2014). Mislabeled or adulterated herbal products can have serious impacts on consumer health through the addition of allergenic or even toxic ingredients (Baker et al. 2012; Posadzki et al. 2012), and further on consumer trust and the reputation of the industry (Wheatley & Spink 2013; O'Connor 2013).

Currently, there is no universal standard practice for authentication of the contents of herbal supplements. Identification of ingredients based on morphology is precluded by the absence of discernable characteristics in dried, powdered, or liquid preparations of many HDS. Many studies have utilized chromatographic methods such as thin-layer chromatography (TLC) and spectroscopic method such as liquid chromatography-mass spectrometry (LC-MS) to authenticate herbal supplements (Vaclavik et al. 2014; Calahan et al. 2016). However, these methods can be limited by the lack of authenticated reference samples (particularly those that account for chemical variability) and further complicated by the presence of multiple ingredients (Kemsley 2013; Upton et al. 2020).

DNA barcoding, a method which uses short DNA sequences to identify a specimen, has been increasingly utilized to authenticate the contents of HDS (Ichim 2019; Grazina et al. 2020). Barcoding can provide unambiguous identification of a specimen and can distinguish between closely related species, although the discriminatory power can vary greatly depending on the taxa of interest and the barcoding region(s) selected. The most commonly used loci are the “official” plant barcodes *rbcL* and *matK* (Hollingsworth et al. 2009), although other loci such as *nrITS*, *trnH-psbA*, and *trnL-trnF* are often utilized depending on the aim and scope of the study (Pettengill & Neel 2010; Li et al. 2011; Pang et al. 2012). A number of studies utilizing barcoding have detected between 4 and 100 percent adulteration and/or substitution among popular HDS (Stoeckle et al. 2011; Baker et al. 2012; Little and Jeanson 2013; Little 2014; Han et al. 2016; Nithaniyal et al. 2017; Gunnels et al. 2020). A major limitation of DNA barcoding, which utilizes Sanger sequencing, is that it is not guaranteed to detect multiple ingredients—co-

amplification of multiple barcode sequences can lead to sequencing failure (Parveen et al. 2016). However, methods such as meta-barcoding, which utilizes “universal” primers and NGS sequencing techniques, can be used to detect and identify multiple ingredients in a HDS (Ivanova et al. 2016; Raclariu et al. 2018). Additionally, the success of DNA barcoding is contingent on the presence of DNA of a sufficient amount and quality. This can vary greatly based on the processing methods which the HDS may experience prior to reaching the market (Ivanova et al. 2016, Grazina et al. 2020).

In early 2015, the New York State Attorney General issued a press release reporting that a DNA barcoding study failed to detect the presence of the labeled ingredients in 71% of tested commercial HDS, including 24 samples of garlic (New York State Attorney General 2015). Although few details were released regarding the design and specific results of study, those within the industry questioned the use of DNA barcoding on samples which may have been subject to DNA degradation as a result of chemical and mechanical processing methods (Harbaugh Reynaud et al. 2015). Use of a mini-barcode (<200 bp) can overcome some of the limitations of traditional barcoding related to DNA quality by targeting a smaller DNA fragment (Ming et al. 2017; Gao et al. 2019).

The *trnL^{UAA}* intron is a region of particular interest for barcoding. As the only Group I intron in the plastid genome, the gene is self-splicing, and thus possesses a highly conserved secondary structure consisting of alternating regions of conserved and variable sequences (Kuhse et al. 1990). The *trnL* gene consists of two exons, with the intervening intron broken down into 12 regions based on secondary structure (Procaccini et al. 1999). The P6 loop region in particular (hereby referred to as *trnL^{UAA}*-P6) has been identified as useful for barcoding (Taberlet et al. 2007; Särkinen et al. 2012) based on its short length, recoverability from degraded samples, and well-tested universal primer sites (Taberlet et al. 2007). Although the discriminatory power of this region is considerably lower than other barcoding regions, due to its short length and constrained primary sequence, it has proven utility in the fields of animal diet analysis (Soininen et al. 2009; Valentini et al. 2009; Kartzin et al. 2015) and HDS authentication (Coghlan et al. 2012; Song et al. 2017; Feng et al. 2021).

The aims of this study are threefold: *i*) to construct and test a *trnL^{UAA}* mini-barcode reference library for *Allium* species; *ii*) to design a *trnL^{UAA}* mini-barcode assay capable of distinguishing *A. sativum* from all other *Allium* species; and *iii*) to estimate the frequency of mislabeled garlic supplements currently available in the US market.

Materials and Methods

Sampling

The *trnL*^{UAA}-P6 reference database was constructed using all *Allium trnL*^{UAA} sequences deposited in GenBank (ncbi.nlm.nih.gov/genbank, accessed August 15, 2020), and additional material collected from voucher specimens. Additionally, sequences for four common filler ingredients were included in the database, following Newmaster et al. (2013). Ten herbarium specimens of *A. sativum* and closely related taxa *A. atrovioleaceum*, *A. dregeanum*, *A. iranicum*, and *A. sphaerocephalon*, were selected as validation samples based on a recent published phylogeny of *Allium* sect. *Allium* (Hirscheegger et al. 2010) (Table S1). The resulting *trnL*^{UAA}-P6 database was aligned using MUSCLE v. 3.8.31 using default parameters (Edgar 2004). Python scripts were used to identify potentially discriminatory nucleotide positions for *A. sativum* compared to other taxa in the reference database. Based on the location of these discriminatory nucleotides, we chose to utilize the “universal” *trnL*^{UAA} reverse primer “h” (Taberlet et al. 2007). However, the region in which the corresponding forward primer “g” is located exhibited slight variation within the reference database. Therefore, a forward primer was designed upstream in a region which was fully conserved, using Primer3 ([Untergasser et al. 2012] default settings with the following modifications: salt correction formula, Schildkraut & Lifson 1965; dNTP and monovalent cation concentrations set based on our PCR buffer). 43 garlic HDS were purchased in New York City area stores and online (Table 1). According to their labels, 24 of these (55.8%) contained garlic bulb powder and 17 (39.5%) contained garlic bulb extracts, as well as one labeled “garlic oil” and one labeled “garlic flakes”. A subset of the supplements labeled as garlic bulb extract listed additional ingredients: rice bran oil ($n = 2$) and soybean oil ($n = 4$).

Molecular Methods

Total DNA was extracted following the procedure in Little (2014). Extracted DNA was quantified using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The *trnL*^{UAA}-P6 mini-barcode was amplified in a 15 μ L volume containing the following: 1.5 μ L 10x PCR buffer (200 mM tris [pH 8.8], 100mM KCl, 100 mM (NH₄)₂SO₄, 20 mM MgSO₄, 1% [v/v] Triton X-100, 50% [w/v] sucrose, 0.25% [w/v] cresol red), 0.2 μ M dNTPs, 0.25 μ g/ μ L BSA, 1.5 μ L DMSO, 0.25 μ M of each primer (5'-AGA CGC TAC GGA CTT GAT TGG A-3' [novel] and 5'-GCT TCC ATT GAG TCT CTG CAC C-3' [Taberlet et al. 2007, primer h]), 1.2 U *Taq* polymerase, and 0.5 μ L purified DNA. Reactions were incubated for 150 s at 95°C, cycled 35 times (30 s at 95°C, 30 s at 68°C, 30 s at 72°C), and finally incubated for 10 min at 72°C.

The full length *trnL-F* sequence was amplified for several voucher specimens as above, with the following modifications: no DMSO, 0.6 M betaine, 0.5 μ M of each primer (5'- CGA AAT CGG TAG ACG CTA CG-3' [Taberlet and Gielly 1991, primer c] and 5'- ATT TGA ACT GGT GAC ACG AG-3' [Taberlet and Gielly 1991, primer f]) and 0.6 U *Taq* polymerase. Reactions were incubated for 150 s at 95°C, cycled 33 times (45 s at 94°C, 120 s at 58°C), and finally incubated for 10 min at 64°C. PCR products were treated with ExoSAP-IT and bidirectionally sequenced on an ABI 3730XL automated using the amplification primers and BigDye v3.1 (Psomagen, New York, USA).

Data Analysis

Contigs were assembled, trimmed, and edited using Sequencher 5.4.6 (GeneCodes Corp, Ann Arbor, MI). Barcode sequence quality (*B*) was calculated with quality threshold (*q*) set to 30 (Little 2010). A multiple sequence alignment was constructed in MUSCLE v. 3.8.31 (Edgar 2004) using the sequenced mini-barcodes and the *trnL*^{UAA}-P6 database. BRONX2 (Little 2012), an alignment-free sequence identification algorithm, was utilized to compare the mini-barcode sequences to the reference database, with the most similar sequence(s) considered a match. Inter- and intra-specific distances were calculated for each taxon in MEGAX (Kumar et al., 2018) using the Kimura-2-parameter (K2P) distance model (Kimura 1980). Minimum interspecific distance was compared with maximum intraspecific distance for the detection of a barcoding gap for *A. sativum* and other economically important *Allium* species (Meier et al. 2008).

Results and Discussion

Reference database

The *trnL*^{UAA}-P6 reference database contains 1619 sequences from 283 *Allium* taxa and 4 common fillers (Table S2), including representatives from all 15 subgenera of *Allium*. There are 116 unique *Allium* haplotypes present in the reference database. The aligned reference database is included in Supplementary File S3. Eight novel full length *trnL-trnF* sequences for *A. dregeanum*, *A. sphaerocephalon*, *A. atrovioleaceum*, and *A. iranicum* (all sect. *Allium*) were generated in this study (Table S1). The *trnL*^{UAA}-P6 region in *Allium* ranges from 79 to 98 bases (mean: 87.7, std. dev: 2.54).

Species discrimination

Based on sequences available in GenBank, neither of the official plant barcoding genes (*rbcL* and *matK*; Hollingsworth et al. 2009) contain suitable mini-barcode candidates for *A. sativum*. In *rbcL*, there are no sites which can individually or in combination uniquely identify *A. sativum*. In *matK*, there are three sites that individually differentiate *A. sativum* from other *Allium*, but the sites are located within variable regions and Primer3 found no acceptable primer pairs given the selected parameters (Figure 1B). However, *trnL*^{UAA}-P6 possesses sufficient discrimination within a short barcode sequence, suitable primer pairs, and an adequate sampling of publicly available reference sequences for comparison. *A. sativum* can be unambiguously differentiated from all other *Allium* in the reference database by three nucleotide positions within *trnL*^{UAA}-P6 (Figure 1A): a ‘T’ at position 56 (versus a ‘G’), a ‘T’ at position 72 (versus a ‘G’), and a ‘G’ at position 87 (versus an ‘A’). A barcoding gap is also observed between the maximum intra- and minimum interspecific distance for *A. sativum* (0 and 0.0257, respectively). Other species of *Allium* listed in Herbs of Commerce (McGuffin et al. 2000), namely *A. cepa* (onion, sect. *Cepa*), *A. schoenoprasum* (chive, sect. *Cepa*), *A. fistulosum* (Welsh onion, sect. *Cepa*), *A. tuberosum* (Chinese chive, sect. *Butomissa*), *A. macrostemon* (long-stamen chive, sect. *Allium*), and *A. ampeloprasum* (leek, sect. *Allium*) cannot be uniquely distinguished using this mini barcode (Figure 1A), and exhibit no barcode gap – in fact, several of these species exhibit greater intra-specific divergence.

BRONX2 was able to correctly identify *A. sativum* validation samples, and to exclude *A. sativum* as a possible identification from validation samples for closely related taxa (*A. sativum* $n = 2$, other *Allium* $n = 8$; sensitivity = 1.0 [95% CI = 0.15–1.0], specificity = 1.0 [95% CI = 0.63–1.0]). Median sequence quality (B_{30}) of the validation sequences was 0.6415 (IQR = 0.607–0.671).

Supplement authentication

The mini-barcode was successfully PCR amplified from 35 of the 43 (81%) HDS sampled. These samples had a median DNA content of 121 ng/μL (IQR 58.55–163.95). The HDS which failed to PCR amplify ($n = 8$) all had a measured DNA content below 50 ng/μL. This included seven samples labeled “garlic extract” as well as the sole garlic oil HDS sampled. A subset of the sampled HDS included additional listed ingredients, specifically carrier oils, but the presence of these did not appear to be directly related to amplification success: 50% with added soybean oil failed ($n = 4$), as did 50% with added rice bran oil ($n = 2$).

Mini-barcode sequences were obtained for 33 of 35 amplified HDS samples (94%), labeled as “garlic powder” [$n = 22$], “garlic extract” [$n = 10$], and “garlic flakes” [$n = 1$]. The samples whose PCR products failed to sequence ($n =$

2) were both labeled “garlic powder”. The median sequence quality (B_{30}) of the supplement barcodes was 0.604 (IQR 0.596–0.611), which is comparable to values obtained in similar studies (Little 2014, median 0.596; Little and Jeanson 2013, median 0.582), and exceed minimum standards suggested by BARCODE Data Standards (Hanner 2012). BRONX2 positively identified each of these HDS samples as *A. sativum* ($n = 33$), which was further confirmed by visual inspection at the three diagnostic positions within the mini-barcode.

The absence of sequences that match other species does not fully preclude the inclusion of off label ingredients, which may be unamplifiable or unrecoverable due to the limits of Sanger sequencing, and the amount and quality of DNA present. Similarly, failure to amplify the barcode region from some of the HDS in this study does not conclusively indicate a lack of *A. sativum* in the sample. Rather, these HDS may not contain a sufficient amount or quality DNA for testing following a variety of processing methods. HDS may also contain a number of excipients (such as binders, pigments, and stabilizers) which may affect the success and quality of DNA extractions (Parveen et al. 2016).

Interestingly, HDS labeled as “garlic extract”, which were likely subject to more extensive processing compared to powdered garlic, saw nearly equal amounts of success and failure ($n = 10$ vs. $n = 8$), indicating that the type of HDS alone does not predict the success of DNA barcoding on the sample, and that success may be highly dependent on the specific processes involved in obtaining these garlic extracts (Novak et al. 2007).

Other techniques such as DNA metabarcoding or chemical analyses may be able to further affirm the presence of *A. sativum*, and the absence of additional off-label ingredients in these samples.

Conclusion

Overall, DNA mini-barcoding is an efficient and accessible method for detection and authentication of products in single-ingredient herbal dietary supplements. The use of full-length barcodes may be unsuitable in cases where DNA is degraded due to processing methods. However, it is demonstrated here that mini-barcodes based on loci such as the *trnL^{UAA}* P6 loop can be used to detect products with relatively high levels of success. The *trnL^{UAA}* P6 loop can unambiguously identify *A. sativum* products, and was successful in detecting garlic within all of the HDS sequenced. It is important to note that the inability to recover DNA from samples of this sort should not be interpreted as confirmation that the ingredient is not present.

These methods may be particularly useful for students and non-scientists to analyze highly processed HDS given limited expertise, materials, and funds. Further work to develop mini-barcode assays for HDS authentication will continue to help improve industry standards and protect consumer health.

Acknowledgments

The authors acknowledge the following: Joan Deutsch and Monica Mimoso for performing DNA extractions and *rbcL/matK* sequencing of reference sequences; Matt Sewell for laboratory support; Royal Botanic Gardens, Kew, and Royal Botanic Garden Edinburgh herbaria for voucher specimens.

Competing interests: The authors declare there are no competing interests.

Contributors' statement: D.P.L. conceived the study and obtained specimens. L.P. performed laboratory procedures and conducted data analysis, D.P.L. and L.P. wrote the manuscript.

Funding: The authors declare no specific funding for this work.

Data availability: Sequences generated in this study are deposited in GenBank as accessions MW377581-MW377584

References

- Abdel-Hafeez, E.H., Ahmad, A.K., Kamal, A.M., Abdellatif, M.Z.M., and Abdelgelil, N.H. 2015. In vivo antiprotozoan effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) extracts on experimentally infected mice with *Blastocystis* spp. *Parasitol. Res.* **114**(9): 3439–3444. doi:10.1007/s00436-015-4569-x.
- Al-Snafi, A.E. 2015. Therapeutic properties of medicinal plants: a review of their effect on reproductive systems. *Indian J. Pharm. Sci. Res.* **5**(4): 240–248.
- American Botanical Council. 2020a. HerbalGram: US sales of herbal supplements increase by 8.6% in 2019. *J. Am. Bot. Counc.* **127**: 54–69. Available from <http://cms.herbalgram.org/herbalgram/issue127/hg127-mktrpt-2019.html>.
- American Botanical Council. 2020b. ABC-AHP-NCNPR Botanical Adulterants Prevention Program. Available from <http://cms.herbalgram.org/BAP/index.html>.
- Ashraf, R., Khan, R.A., and Ashraf, I. 2011. Garlic (*Allium sativum*) supplementation with standard antidiabetic agent provides better diabetic control in type 2 diabetes patients. *Pak. J. Pharm. Sci.* **24**(4): 565–570.
- Baker, D., Stevenson, D., and Little, D. 2012. DNA Barcode identification of black cohosh herbal dietary supplements. *J. AOAC Int.* **95**(4): 1023–1034. doi:10.5740/jaoacint.11-261.
- Batiha, E.-S., Gaber, Amany Magdy Beshbishy, L.G.W., Elewa, Y.H., Al-Sagan, A.A., El-Hack, A., Mohamed, E., Taha, A.E., Abd-Elhakim, Y.M., and Devkota, H.P. 2020. Chemical constituents and pharmacological activities of garlic : a review. *Nutrients* **12**(3): 872. doi:10.3390/nu12030872.
- Calahan, J., Howard, D., Almalki, A.J., Gupta, M.P. and Calderón, A.I., 2016. Chemical adulterants in herbal medicinal products: a review. *Planta Med.* **82**(6): 505-515. doi: 10.1055/s-0042-103495.
- Choi, I.S., Cha, H.S., and Lee, Y.S. 2014. Physicochemical and antioxidant properties of black garlic. *Molecules* **19**(10): 16811–16823. doi:10.3390/molecules191016811.
- Chung, L.Y. 2006. The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. *J. Medicinal Food* **9**(2): 205–213. doi:10.1089/jmf.2006.9.205
- Coghlan, M., Haile, J., Houston, J., Murray, D., White, N., Moolhuijzen, P., Bellgard, M., and Bunce, M. 2012. Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. *PLoS Genet.* **8**(4): e1002657. doi:10.1371/journal.pgen.1002657.

- 240 Davis, S.R. 2005. An overview of the antifungal properties of allicin and its breakdown products - The possibility of
241 a safe and effective antifungal prophylactic. *Mycoses* **48**(2): 95–100. doi:10.1111/j.1439-0507.2004.01076.x.
- 242 Dubey, H., Singh, A., Patole, A.M., and Tenpe, C.R. 2017. Antihypertensive effect of allicin in dexamethasone-
243 induced hypertensive rats. *Integr. Med. Res.* **6**(1): 60–65. doi:10.1016/j.imr.2016.12.002.
- 244 Edgar, R. 2004. MUSCLE : multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids*
245 *Res.* **32**(5): 1792–1797. doi:10.1093/nar/gkh340.
- 246 Feng, T.T., Qiu, S.M., Tan, G.L., Tian, D.M., Wu, D.M., Zhou, L., Yang, M.X., and Zhu, S. 2021. Identification of
247 suitable barcodes for specifically detecting adulterants of turnip (*Brassica rapa*) and radish (*Raphanus sativus*)
248 in maca (*Lepidium meyenii*). *Food. Anal. Method.* **14**: 66-73. doi:10.1007/s12161-020-01857-6 .
- 249 Gao, Z., Liu, Y., Wang, X., Wei, X. and Han, J., 2019. DNA mini-barcoding: a derived barcoding method for herbal
250 molecular identification. *Frontiers in plant science*, **10**: 987. doi: 10.3389/fpls.2019.00987.
- 251 Garcia-Alvarez, A., Egan, B., De Klein, S., Dima, L., Maggi, F.M., Isoniemi, M., Ribas-Barba, L., Raats, M.M.,
252 Meissner, E.M., Badea, M., Bruno, F., Salmenhaara, M., Milà-Villarroel, R., Knaze, V., Hodgkins, C.,
253 Marculescu, A., Uusitalo, L., Restani, P., and Serra-Majem, L. 2014. Usage of plant food supplements across
254 six European countries: Findings from the PlantLIBRA consumer survey. *PLoS One* **9**(3).
255 doi:10.1371/journal.pone.0092265.
- 256 Grazina, L., Amaral, J.S. and Mafra, I., 2020. Botanical origin authentication of dietary supplements by DNA-based
257 approaches. *Comprehensive Reviews in Food Science and Food Safety*, **19**(3): 1080-1109. doi: 10.1111/1541-
258 4337.12551
- 259 Gunnels, T., Creswell, M., McFerrin, J. and Whittall, J.B., 2020. The ITS region provides a reliable DNA barcode
260 for identifying reishi/lingzhi (*Ganoderma*) from herbal supplements. *PLoS One*, **15**(11): e0236774. doi:
261 10.1371/journal.pone.0236774
- 262 Han, J., Pang, X., Liao, B., Yao, H., Song, J., and Chen, S. 2016. An authenticity survey of herbal medicines from
263 markets in China using DNA barcoding. *Sci. Rep.* **6**(January): 1–9. doi:10.1038/srep18723.
- 264 Hanner, R., and the Consortium for the Barcode of Life 2012. Data standards for BARCODE records in INSDC
265 (BRIs). Available from: <https://repository.si.edu/handle/10088/96518>.

- Harbaugh Reynaud, D.T., Mishler, B.D., Neal-Kababick, J., and Brown, P.N. 2015. The capabilities and limitations of DNA barcoding of botanical dietary supplements. [White paper]. Available from: http://www.ahpa.org/Portals/0/PDFs/The-Capabilities-and-Limitations-of-DNA-Testing-FINAL_AHPA.pdf
- Hirayama, F., Lee, A.H., Binns, C.W., Watanabe, F., and Ogawa, T. 2008. Dietary supplementation by older adults in Japan. *Asia Pac. J. Clin. Nutr.* **17**(2): 280–284. doi:10.6133/apjcn.2008.17.2.15.
- Hirscheegger, P., Jakše, J., Trontelj, P., and Bohanec, B. 2010. Origins of *Allium ampeloprasum* horticultural groups and a molecular phylogeny of the section *Allium* (*Allium*: Alliaceae). *Mol. Phylogenet. Evol.* **54**(2): 488–497. doi:10.1016/j.ympev.2009.08.030.
- Hollingsworth, P., Forrest, L., Spouge, J., Hajibabaei, M., Ratnasingham, S., van der Bank, M., Chase, M., Cowan, R., Erickson, D., Fazekas, A., Graham, S., James, K., Kim, K.-J., Kress, W., Schneider, H., van AlphenStahl, J., Barrett, S., van den Berg, C., Bogarin, D., Burgess, K., Cameron, K., Carine, M., Chacon, J., Clark, A., Clarkson, J., Conrad, F., Devey, D., Ford, C., Hedderson, T., Hollingsworth, M., Husband, B., Kelly, L., Kesanakurti, P., Kim, J., Kim, Y.-D., Lahaye, R., Lee, H.-L., Long, D., Madrinan, S., Maurin, O., Meusnier, I., Newmaster, S., Park, C.-W., Percy, D., Petersen, G., Richardson, J., Salazar, G., Savolainen, V., Seberg, O., Wilkinson, M., Yi, D.-K., and Little, D. 2009. A DNA barcode for land plants. *PNAS* **106**(31): 12794–7. doi:10.1073/pnas.0905845106.
- Hussein, H.J., Hameed, I.H., and Hadi, M.Y. 2017. A review: Anti-microbial, anti-inflammatory effect and cardiovascular effects of garlic: *Allium sativum*. *Res. J. Pharm. Technol.* **10**(11): 4069–4078. doi:10.5958/0974-360X.2017.00738.7.
- Ichim, M.C., 2019. The DNA-based authentication of commercial herbal products reveals their globally widespread adulteration. *Frontiers in pharmacology*, **10**: 1227. doi: 10.3389/fphar.2019.01227
- Ivanova N.V., Kuzmina M.L., Braukmann T.W.A., Borisenko A.V., and Zakharov E.V. 2016. Authentication of herbal supplements using next-generation sequencing. *PLoS ONE* **11**(5): e0156426. doi: 10.1371/journal.pone.0156426
- Kartzinel, T.R., Chen, P.A., Coverdale, T.C., Erickson, D.L., Kress, W.J., Kuzmina, M.L., Rubenstein, D.I., Wang, W., and Pringle, R.M. 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proc. Natl. Acad. Sci. U. S. A.* **112**(26): 8019–8024. doi:10.1073/pnas.1503283112.
- Kemsley, J. 2013. Analyzing botanical dietary supplements. *Chem. Eng. News* **91**(11).

- Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, **16**(2): 111-120.
- Kuhse, M.G., Strickland, R., and Palmer, J.D. 1990. An ancient group I intron shared by eubacteria and chloroplasts. *Science*. **250**(4987): 1570–1573. doi:10.1126/science.2125748.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, **35**(6): 1547-1549. doi: 10.1093/molbev/msy096.
- Li, D.Z., Gao, L.M., Li, H.T., Wang, H., Ge, X.J., Liu, J.Q., Chen, Z.D., Zhou, S.L., Chen, S.L., Yang, J.B., Fu, C.X., Zeng, C.X., Yan, H.F., Zhu, Y.J., Sun, Y.S., Chen, S.Y., Zhao, L., Wang, K., Yang, T., and Duan, G.W. 2011. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proc. Natl. Acad. Sci. U. S. A.* **108**(49): 19641–19646. doi:10.1073/pnas.1104551108.
- Li, W.R., Shi, Q.S., Liang, Q., Huang, X.M., and Chen, Y. Ben. 2014. Antifungal effect and mechanism of garlic oil on *Penicillium funiculosum*. *Appl. Microbiol. Biotechnol.* **98**(19): 8337–8346. doi:10.1007/s00253-014-5919-9.
- Little, D.P.. 2010. A unified index of sequence quality and contig overlap for DNA barcoding. *Bioinformatics* **26**(21): 2780–2781. doi:10.1093/bioinformatics/btq507.
- Little, D.P. 2012. BRONX2: Barcode Recognition Obtained with Nucleotide eXposes. <https://www.nybg.org/files/scientists/dlittle/BRONX2.html>
- Little, D.P. 2014. Authentication of *Ginkgo biloba* herbal dietary supplements using DNA barcoding. *Genome* **57**(9): 513–516. doi:10.1139/gen-2014-0130.
- Little, D.P., and Jeanson, M.L. 2013. DNA barcode authentication of saw palmetto herbal dietary supplements. *Sci. Rep.* **3**: 1–6. doi:10.1038/srep03518.
- Liu, Y., Yan, J., Han, X., and Hu, W. 2015. Garlic-derived compound S-allylmercaptocysteine (SAMC) is active against anaplastic thyroid cancer cell line 8305C (HPACC). *Technol. Heal. Care* **23**(S1): S89–S93. doi:10.3233/thc-150936.
- Low, T.Y., Wong, K.O., Yap, A.L.L., De Haan, L.H.J., and Rietjens, I.M.C.M. 2017. The regulatory framework across international jurisdictions for risks associated with consumption of botanical food supplements. *Compr. Rev. Food Sci. Food Saf.* **16**(5): 821–834. doi:10.1111/1541-4337.12289.

- 322 Martins, N., Petropoulos, S., and Ferreira, I.C.F.R. 2016. Chemical composition and bioactive compounds of garlic
323 (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chem. **211**: 41–50.
324 doi:10.1016/j.foodchem.2016.05.029.
- 325 McGuffin, M., Karesz, J., Leung, A., and Tucker, A. 2000. *Herbs of Commerce*. Second Edition. AHPA.
- 326 Meier, R., Zhang, G. and Ali, F., 2008. The use of mean instead of smallest interspecific distances exaggerates the
327 size of the “barcoding gap” and leads to misidentification. Syst. Biol., **57**(5): 809-813. doi:
328 10.1080/10635150802406343.
- 329 Messerer, M., Johansson, S.-E., and Wolk, A. 2001. Use of natural remedies and dietary supplements increased
330 dramatically during the 1980s and the 1990s. J. Intern. Med. **250**(10): 160–166. doi: 10.1046/j.1365-
331 2796.2001.00872.x
- 332 Ming, S., Gang-Qiang, D., Zhang, Y.Q., Xia, L. and Wei, S. 2017. Identification of processed Chinese medicinal
333 materials using DNA mini-barcoding. Chinese journal of natural medicines, **15**(7): 481-486. doi:
334 10.3724/SP.J.1009.2017.00481.
- 335 New York State Attorney General. 2015, 3 February. A.G. Schneiderman Asks Major Retailers To Halt Sales Of
336 Certain Herbal Supplements As DNA Tests Fail To Detect Plant Materials Listed On Majority Of Products
337 Tested [Press release]. Available at: [https://ag.ny.gov/press-release/2015/ag-schneiderman-asks-major-](https://ag.ny.gov/press-release/2015/ag-schneiderman-asks-major-retailers-halt-sales-certain-herbal-supplements-dna)
338 [retailers-halt-sales-certain-herbal-supplements-dna](https://ag.ny.gov/press-release/2015/ag-schneiderman-asks-major-retailers-halt-sales-certain-herbal-supplements-dna).
- 339 Newmaster, S., Grguric, M., Shanmughanandhan, D., Ramalingam, S., and Ragupathy, S. 2013. DNA barcoding
340 detects contamination and substitution in North American herbal products. BMC Med. **11**(222).
341 doi:10.1016/j.sajb.2017.01.162.
- 342 Nguyen, N.H., Driscoll, H.E., and Specht, C.D. 2008. A molecular phylogeny of the wild onions (*Allium*; Alliaceae)
343 with a focus on the western North American center of diversity. Mol. Phylogenet. Evol. **47**(3): 1157–1172.
344 doi:10.1016/j.ympev.2007.12.006.
- 345 Nithaniyal, S., Vassou, S.L., Poovitha, S., Raju, B. and Parani, M., 2017. Identification of species adulteration in
346 traded medicinal plant raw drugs using DNA barcoding. Genome, **60**(2): 139-146. doi: 10.1139/gen-2015-
347 0225.
- 348 Nouroz, F., Mehboob, M., Noreen, S., Zaidi, F., and Mobin, T. 2015. A review on anticancer activities of garlic (
349 *Allium sativum* L.). Middle East J. Sci., **23**(6): 1145–1151. doi:10.5829/idosi.mejsr.2015.23.06.9381.

- 350 Novak, J., Grausgruber-Gröger, S. and Lukas, B., 2007. DNA-based authentication of plant extracts. Food Res.
351 Int., **40**(3): 388–392. doi: 10.1016/j.foodres.2006.10.015.
- 352 O'Connor, A. 2013, November 3. Herbal supplements are often not what they seem. New York Times.
- 353 Orengo, K.O., Maitho, T., Mbaria, J.M., Maingi, N., and Kitaa, J.M. 2016. In vitro anthelmintic activity of *Allium*
354 *sativum*, *Allium cepa* and *Jatropha curcas* against *Toxocara canis* and *Ancylostoma caninum*. African J.
355 Pharm. Pharmacol. **10**(21): 465–471. doi:10.5897/ajpp2016.4551.
- 356 Padiya, R., and K. Banerjee, S. 2013. Garlic as an anti-diabetic agent: Recent progress and patent reviews. Recent
357 Pat. Food. Nutr. Agric. **5**(2): 105–127. doi:10.2174/18761429113059990002.
- 358 Pang, X., Liu, C., Shi, L., Liu, R., Liang, D., Li, H., Cherny, S.S., and Chen, S. 2012. Utility of the *trnH-psbA*
359 intergenic spacer region and its combinations as plant DNA barcodes: A Meta-Analysis. PLoS One **7**(11): 1–9.
360 doi:10.1371/journal.pone.0048833.
- 361 Parveen, I., Gafner, S., Techen, N., Murch, S.J. and Khan, I.A., 2016. DNA barcoding for the identification of
362 botanicals in herbal medicine and dietary supplements: strengths and limitations. Planta Medica, **82**(14): 1225-
363 1235. doi: 10.1055/s-0042-111208.
- 364 Pettengill, J., and Neel, M. 2010. An evaluation of candidate plant DNA barcodes and assignment methods in
365 diagnosing 29 species in the genus *Agalinis* (Orobanchaceae). Am. J. Bot. **97**(8): 1391–1406.
366 doi:10.3732/ajb.0900176.
- 367 Posadzki, P., Watson, L., and Ernst, E. 2012. Contamination and adulteration of herbal medicinal products (HMPs):
368 An overview of systematic reviews. Eur. J. Clin. Pharmacol. **69**(3): 295–307. doi:10.1007/s00228-012-1353-z.
- 369 Prasad Giri, R., Gangawane, A.K., and Ghorai Giri, S. 2008. Regulation on herbal product used as medicine around
370 the world: a review. Int. Res. J. Eng. Technol. **217**: 217–223.
- 371 Procaccini, G., Mazzella, L., Alberte, R.S., and Les, D.H. 1999. Chloroplast tRNA(Leu) (UAA) intron sequences
372 provide phylogenetic resolution of seagrass relationships. Aquat. Bot. **62**(4): 269–283. doi:10.1016/S0304-
373 3770(98)00099-0.
- 374 Raclariu, A., Heinrich, M., Ichim, M., and de Boer, H. 2018. Benefits and limitations of DNA barcoding and
375 metabarcoding in herbal product authentication. Phytochem. Anal. **29**(2): 123–128. doi:10.1002/pca.2732.
- 376 Rahman, M.M., Fazlic, V., and Saad, N.W. 2012. Antioxidant properties of raw garlic (*Allium sativum*) extract. Int.
377 Food Res. J. **19**(2): 589–591.

- 378 Ried, K., and Fakler, P. 2014. Potential of garlic (*Allium sativum*) in lowering high blood pressure: Mechanisms of
379 action and clinical relevance. *Integr. Blood Press. Control* **7**: 71–82. doi:10.2147/IBPC.S51434.
- 380 Rohner, A., Ried, K., Sobenin, I.A., Bucher, H.C., and Nordmann, A.J. 2015. A systematic review and metaanalysis
381 on the effects of garlic preparations on blood pressure in individuals with hypertension. *Am. J. Hypertens.*
382 **28**(3): 414–423. doi:10.1093/ajh/hpu165.
- 383 Särkinen, T., Staats, M., Richardson, J.E., Cowan, R.S., and Bakker, F.T. 2012. How to open the treasure chest?
384 Optimising DNA extraction from herbarium specimens. *PLoS One* **7**(8): 1–9.
385 doi:10.1371/journal.pone.0043808.
- 386 Schildkraut, C. and Lifson, S., 1965. Dependence of the melting temperature of DNA on salt
387 concentration. *Biopolymers: Original Research on Biomolecules*, **3**(2): 195-208.
- 388 Seki, T., and Ariga, T. 2006. Antithrombotic and anticancer effects of garlic-derived sulfur compounds: a review.
389 *Biofactors* **26**: 93–103. doi:10.1002/biof.5520260201.
- 390 Singh, S., and Levine, M. 2006. Natural health product use in Canada: Analysis of the National Population Health
391 Survey. *J. Popul. Ther. Clin. Pharmacol.* **13**(2).
- 392 Soininen, E., Valentini, A., Coissac, E., Miquel, C., Gielly, L., Brochmann, C., Brysting, A., Sønstebo, J., Ims, R.,
393 Yoccoz, N., and Taberlet, P. 2009. Analysing diet of small herbivores: The efficiency of DNA barcoding
394 coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures.
395 *Front. Zool.* **6**(1): 1–9. doi:10.1186/1742-9994-6-16.
- 396 Song, M., Dong, G., Zhang, Y., Liu, X., and Sun, W. 2017. Identification of processed Chinese medicinal materials
397 using DNA mini-barcoding. *Chin. J. Nat. Med.* **15**(7): 481–486. doi:10.1016/S1875-5364(17)30073-0.
- 398 Soininen, E., Valentini, A., Coissac, E., Miquel, C., Gielly, L., Brochmann, C., Brysting, A., Sønstebo, J., Ims, R.,
399 Yoccoz, N., and Taberlet, P. 2009. Analysing diet of small herbivores: The efficiency of DNA barcoding
400 coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures.
401 *Front. Zool.* **6**(1): 1–9. doi:10.1186/1742-9994-6-16.
- 402 Stoeckle, M., Gamble, C., Kirpekar, R., Young, G., Ahmed, S., and Little, D. 2011. Commercial teas highlight plant
403 DNA barcode identification successes and Obstacles. *Nat. Sci. Reports* **1**: 1–7. doi:10.1038/srep00042.

- 404 Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann,
 405 C., and Willerslev, E. 2007. Power and limitations of the chloroplast *trnL* (UAA) intron for plant DNA
 406 barcoding. *Nucleic Acids Res.* **35**(3). doi:10.1093/nar/gkl938.
- 407 Taberlet, P., Gielly, L., Pautou, G., and Bouvet, J. 1991. Universal primers for amplification of three noncoding
 408 regions of chloroplast DNA. *Plant Mol. Biol.* **17**: 1105–1109. doi:10.1007/BF00037152.
- 409 Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B., Remm, M., and Rozen, S. 2012. Primer3--new
 410 capabilities and interfaces. *Nucleic Acids Res.* **40**(15). doi:10.1093/nar/gks596.
- 411 Upton, R., David, B., Gafner, S., and Glasl, S. 2020. Botanical ingredient identification and quality assessment:
 412 strengths and limitations of analytical techniques. *Phytochem. Rev.* **19**:1157-1177. doi:10.1007/s11101-019-
 413 09625-z.
- 414 Vaclavik, L., Krynnitsky, A., and Rader, J. 2014. Mass spectrometric analysis of pharmaceutical adulterants in
 415 products labeled as botanical dietary supplements or herbal remedies: A review. *Anal. Bioanal. Chem.*
 416 **406**(27): 6767–6790. doi:10.1007/s00216-014-8159-z.
- 417 Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti,
 418 G., Wincker, P., Swenson, J.E., and Taberlet, P. 2009. New perspectives in diet analysis based on DNA
 419 barcoding and parallel pyrosequencing: The *trnL* approach. *Mol. Ecol. Resour.* **9**(1): 51–60.
 420 doi:10.1111/j.1755-0998.2008.02352.x.
- 421 Wheatley, V.M., and Spink, J. 2013. Defining the public health threat of dietary supplement fraud. *Compr. Rev.*
 422 *Food Sci. Food Saf.* **12**(6): 599–613. doi:10.1111/1541-4337.12033.
- 423 Wu, C.H., Wang, C.C., Tsai, M.T., Huang, W.T., and Kennedy, J. 2014. Trend and pattern of herb and supplement
 424 use in the United States: Results from the 2002, 2007, and 2012 national health interview surveys. *Evidence-*
 425 *based Complement. Altern. Med.* **2014**. Hindawi Publishing Corporation. doi:10.1155/2014/872320.
- 426 Zhai, B., Zhang, C., Sheng, Y., Zhao, C., He, X., Xu, W., Huang, K., and Luo, Y. 2018. Hypoglycemic and
 427 hypolipidemic effect of S-allyl-cysteine sulfoxide (alliin) in DIO mice. *Sci. Rep.* **8**(1): 1–7.
 428 doi:10.1038/s41598-018-21421-x.

Table 1. Garlic herbal dietary supplements analyzed in this study. Price point: \$ = ≤\$10; \$\$ = 10<x<20; \$\$\$ = ≥\$20.

Sample Name	Supplement Type	Additional Listed Ingredients	Price Point	# of capsules/tablets	Successful PCR?	Successful sequencing?
AS01	powder		\$\$	90	Y	Y
AS02	powder		\$\$	100	Y	Y
AS03	powder		\$	120	Y	Y
AS04	powder		\$	100	Y	Y
AS05	extract		\$\$	200	N	N
AS06	extract	soybean oil	\$	100	Y	Y
AS07	powder		\$\$	60	Y	Y
AS08	extract	soybean oil	\$	100	Y	Y
AS09	extract	flaxseed oil sunflower lecithin	\$\$	90	N	N
AS10	oil		\$	n/a	N	N
AS11	powder		\$\$\$	100	Y	Y
AS12	powder	millet	\$\$	100	Y	Y
AS13	powder		\$\$\$	300	Y	Y
AS14	extract		\$\$	100	Y	Y
AS15	powder		\$	100	Y	Y
AS16	powder		\$	100	Y	Y
AS17	powder		\$	120	Y	Y
AS18	powder		\$\$	90	Y	Y
AS19	extract		\$	90	N	N
AS20	extract		\$\$\$	60	Y	Y
AS21	powder		\$\$\$	120	Y	Y
AS22	flakes		\$	n/a	Y	Y
AS23	powder		\$	n/a	Y	Y
AS24	extract	soybean oil	\$	120	N	N
AS25	extract	soybean oil	\$	100	N	N
AS26	extract		\$\$	90	Y	Y
AS27	powder		\$\$	90	Y	Y
AS28	extract	rice bran oil	\$\$	180	Y	N
AS29	extract	rice bran oil	\$\$	180	N	N
AS30	extract		\$\$	60	Y	Y
AS31	powder		\$	100	Y	Y
AS32	powder		\$\$	60	Y	Y
AS33	extract		\$\$	200	Y	Y
AS34	powder		\$	100	Y	N
AS35	powder		\$	90	Y	Y
AS36	extract		\$	30	Y	Y
AS37	powder		\$	100	Y	Y
AS38	extract		\$	60	Y	Y
AS39	powder		\$	60	Y	Y
AS40	powder		\$\$\$	60	Y	Y
AS41	extract		\$\$	100	N	N

AS42	powder		\$	40	Y	Y
AS43	powder		\$	120	Y	Y

Draft

- 1 Figure 1. (A) Diagnostic nucleotide positions for *Allium sativum* within *trnL*^{UAA}-P6, compared to other commercially
2 important *Allium* taxa and all *Allium* subgenera. Positions are numbered vertically in reference to the aligned
3 *trnL*^{UAA}-P6 reference database. The number of sequences summarized for each taxon is indicated in column “*n*”. (B)
4 Diagnostic nucleotide positions for *Allium sativum* within *matK*, compared to available *Allium* subgenera sequences.
5 Positions are numbered in reference to alignment of complete *matK* sequences.

Draft

A		000
		578
	<i>n</i>	627
<i>Allium sativum</i>	3	TTG
<i>A. macrostemon</i>	12	GGA
<i>A. tuberosum</i>	16	GGA
<i>A. ampeloprasum</i>	12	GGA
<i>A. cepa</i>	8	GGA
<i>A. fistulosum</i>	3	GRA
<i>A. schoenoprasum</i>	5	GGA
<i>A. tuberosum</i>	10	GKA
sect. <i>Allium</i>	73	GGR
sect. <i>Amerallium</i>	219	GKA
sect. <i>Anguinum</i>	8	GGA
sect. <i>Butomissa</i>	3	GGA
sect. <i>Caloscordum</i>	2	GGA
sect. <i>Cepa</i>	23	GRA
sect. <i>Cyathophora</i>	56	GGA
sect. <i>Melanocrommyum</i>	376	GGA
sect. <i>Microscordum</i>	2	GGA
sect. <i>Nectaroscordum</i>	2	GGA
sect. <i>Polyprason</i>	17	GGA
sect. <i>Porphyroprason</i>	3	GGA
sect. <i>Reticulatobulbosa</i>	722	GGA
sect. <i>Rhizirideum</i>	17	GGA
sect. <i>Vvedenskya</i>	3	GGA

B		011
		255
	<i>n</i>	115
<i>Allium sativum</i>	3	AGT
sect. <i>Allium</i>	12	CAC
sect. <i>Amerallium</i>	5	CAC
sect. <i>Anguinum</i>	10	CAC
sect. <i>Butomissa</i>	3	CAC
sect. <i>Caloscordum</i>	1	CAC
sect. <i>Cepa</i>	21	CAC
sect. <i>Cyathophora</i>	7	CAC
sect. <i>Melanocrommyum</i>	1	CAC
sect. <i>Microscordum</i>	1	CAC
sect. <i>Polyprason</i>	7	CAC
sect. <i>Reticulatobulbosa</i>	3	CAC
sect. <i>Rhizirideum</i>	4	CAC

6

7